FACULTEIT
BIO-INGENIEURSWETENSCHAPPEN

INCLUSION OF BLACK SOLDIER FLY (*HERMETIA ILLUCENS*) LARVAE MEAL IN THE DIET FOR LAYING QUAILS: EFFECT ON LIVE PERFORMANCES AND EGGS QUALITY

Aantal woorden: 17.043

Lotte Decraene
Stamnummer: 01270103

Promotor: Dr. ir. Joris Michiels
Copromotor: Prof. dr. Antonella Dalle Zotte

Masterproef voorgelegd voor het behalen van de graad in de richting Master of Science in de biowetenschappen: land- en tuinbouwkunde

Academiejaar: 2016 - 2017

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Foreword

The past year was a great experience, I had the opportunity to work on a very interesting animal trial abroad. I did not only learn a lot about quails, I also learned how to conduct an animal trial, about lab techniques and how to make animal feed, which I barely knew something about. And on top of all that, I got a taste of the Italian life.

At first I want to thank Dr. Ir. Joris Michiels and Dr. Prof. Antonella Dalle Zotte for giving me the opportunity to research this interesting topic in a international environment, for reading and correcting my texts, for helping me when needed. This was an unforgetable experience where I learned a great deal.
A big ‘thank you’ goes out to Marco and Giulia for all the help and the huge amount of work they did on the data, with the quails and in the lab.
I also want to thank Elisabetta, Sandro, Massimo and Lorenzo for showing me how to analyse the quail eggs in the lab and for helping me with this.
Thank you to Leen for re-reading the text.
In last place I want to thank my parents for supporting me about going abroad, for all the help with administrative stuff and for re-reading my text.

Abstract

Insects are an appealing ingredient for animal feed because of the many advantages (environmental, economic and nutritional) they might offer. They can be grown on smaller areas than conventional feed ingredients, have a short reproduction cycle and can be fed with organic waste streams. Currently, it is not allowed to process insects in animal feed, but a change in this regulation is expected.

One insect that can be considered to use as a feed ingredient is the black soldier fly (BSF), *Hermetia illucens*. Previous studies showed the BSF is a suitable ingredient for poultry feed.

This thesis studies the effect of increasing levels of BSF on the egg quality and live performances of quails. In the experiment 225 quails got subdivided in 3 groups. The control (C) group got a diet with an inclusion of 0% BSF; the diets of the H1 and H2 group contained respectively 10 and 15% BSF. The quails were divided in cages of 15 quails, each treatment had 6 cages. After 3 weeks of adaptation, the eggs were collected for one week. A sensory, physical and chemical analysis was executed to determine the egg quality as well as a shelf-life trial. The initial and final weight of the quails were determined, the laying performance and the mortality.

A similar egg quality was found in the C, H1 and H2 group. The H2 diet resulted in a higher egg ash level, but a lower protein level. Dry matter (DM) and cholesterol level showed no difference between treatments. The lipid level was equal between groups but the fatty acid profile was different. All the eggs did not deteriorate after 28 days, the eggs have a long shelf-life.

There was no difference in life performances between groups, although a high mortality in quails with a low bodyweight was found in H1. Also in one cage in the H2 group was a low amount of layed eggs. These findings could not be explained.

It was concluded that the BSF is a good feed ingredient for laying quails up to 15%.

Keywords: black soldier fly, quail, feed, egg quality, live performances

Insecten zijn een aantrekkelijk ingrediënt voor dierenvoeder door de vele mogelijke voordelen (milieu, economisch en nutritioneel). Ze kunnen gekweekt worden op kleinere oppervlaktes dan conventionele voederingrediënten; ze hebben een korte reproductiecyclus en kunnen met organische afvalstromen gevoerd worden. Momenteel is het niet toegelaten om insecten te verwerken in dierenvoeders; echter verwacht men een verandering in deze wet.
Een te overwegen insect is de zwarte soldatenvlieg (BSF), *Hermetia illucens*. Eerdere studies toonden aan dat de BSF een geschikt ingrediënt voor voeder voor gevogelte is.
Deze thesis onderzoekt het effect van toenemende levels BSF op de eikwaliteit en de levensprestaties van kwartels.
In het experiment werden 180 kwartels onderverdeel in 3 groepen: de controle (C) groep kreeg een dieet met 0% inclusie van BSF, de diëten van de H1 en H2 groep bevatten respectievelijk 10 en 15% BSF. Na 3 weken adaptatie, werd gedurende 1 week een collectie van de eieren uitgevoerd. Een sensorische, fysische en chemische analyse en een houdbaarheidsproef werden uitgevoerd om de eikwaliteit te bepalen. Het begin- en eindgewicht van de kwartels, de legprestatie en de mortaliteit werden bepaald.
Er werd een gelijkaardige eikwaliteit gevonden in alle groepen. Het H2 dieet resulteerde in een hoger as- maar een lager proteïnengehalte. Er werd geen verschil in DM en cholesterol tussen de eieren van verschillende behandelingen gevonden. Het vetgehalte tussen de groepen was gelijk, maar het vetzurenprofiel was verschillend. Alle eieren waren niet afgetakeld in kwaliteit na 28 dagen, de eieren hebben een lange houdbaarheidsdatum.
Tussen de groepen werd er geen verschil in levensprestaties gevonden, maar een hoge mortaliteit met een laag lichaamsgewicht werd gedetecteerd in H1. In één kooi van de H2 groep werd er een lage hoeveelheid gelegde eieren vastgesteld. Deze bevindingen konden niet worden verklaard.
Er werd besloten dat de BSF een goed ingrediënt is voor voeder van kwartels tot een inclusie van 15%.

Kernwoorden: zwarte soldatenvlieg, kwartel, voeder, eierkwaliteit, levensprestaties

Content

List with abreviations

ADF	Acid detergent fiber
AIDC	Apparent ileal digestiblity coefficients
AMP	Antimicrobial peptides
avP	Available P
BHT	Butylhydroxytolueen
BSF	Black soldier fly
C	Control diet (0% inclusion of BSF)
CCTAD	Apparent digestibility coefficients of the total tract
CF	Crude fiber
CL	Control line
DHA	Docosahexaenoic acid
DM	Dry matter
EPA	Eicosapentaenoic acid
FA	Fatty acid
H1	Diet with 10% inclusion of BSF
H2	Diet with 15% inclusion of BSF
HDL-C	High density lipoprotein cholesterol
HL	High body weight line
LDL-C	Low density lipoprotein cholesterol
LL	Low body weight line
LL	Layer line
MDA	Malonaldehyde
MUFA	Monounsaturated fatty acid
NDF	Neutral detergent fiber
PTTH	Prothoracicotrop hormone
PUFA	Polyunsaturated fatty acid
SFA	Saturated fatty acid
STD	Standard
TBARS	Thiobarbituric acid reactive substances
TCA	Trichloroacetic acid

List with tables and figures

1. Introduction

This thesis studies the effect of BSF in the diet of laying quails. Any change in live performances (weight, laying performance and mortality) are investigated as well as the egg quality (sensory and physico-chemical quality and the shelf-life).

Poultry requires a high quantity and quality protein diet. Because of the increasing prices and the environmental disadvantages (land occupation, energy and water use) that comes with the use of soybean meal, an alternative protein source has to be searched.
Insect meal could provide an answer. Economical, nutritional and environmental advantages make insects an appealing source of protein.
Insects can be reared in smaller areas without a big use of water. They can be fed with organic waste streams, converting them into high valuable protein, hereby waste is being reduced and livestock feed is produced. These efficient food converters produce a high quality and quantity protein which are rich in essential amino acids. Because of their short reproduction cycle, a large number of insects can be produced in a short amount of time.

The BSF, *Hermetia illucens*, can replace conventional feed ingredients for poultry like fishmeal and soybean meal, which have a high environmental impact. Furthermore, insects belong to the natural diet of poultry (De Marco, Martinez et al., 2015).
The BSF belongs to the order Diptera and the family Stratiomyidae (Tomberlin, Sheppard et al., 2002). The development of the BSF consists of four stages: egg, larvae, pupae and adult. The larval stage lasts 14 days or longer and involves several moltings, finally resulting in the prepupal stage. At this stage, the larvae gets rid of its digestive tract and migrates to a place to pupate (Sheppard, Newton et al., 1994). The pupal stage lasts 10-14 days (Sheppard, Tomberlin et al., 2002). Adults mate 2 days after emergence and the females lay eggs 2 days afterwards. Four days later the eggs hatch (Tomberlin & Sheppard, 2002). The larvae go through 5 instars and feed themselves on a variety of products going from faeces (Myers, Tomberlin et al., 2008) to kitchen waste (Nguyen, Tomberlin et al., 2015). The BSF only consumes organic matter in the larval stage. The larvae eat as much as possible to support their metabolism during the pupal and adult stage (Newton et al., 2005).

At this moment, the use of insects in animal feed is forbidden in the European Union. Because of all the advantages insects might offer, a lot of research is conducted nowadays on this subject. A change in the EU-regulation is expected in the future.

To obtain background knowlegde of this subject, a literature study is made. The nutritional value and the production of BSF are discussed as well as the regulations about the use of BSF in animal feed. Later on, information is given about the farming of laying quails and egg quality.

For the research itself an experimental trial is done. 225 quails are given 3 different diets: control diet with 0% BSF, H1 diet with 10% BSF and H2 diet with 15% BSF. A chemical analysis on these diets is done. The eggs of the quails are collected and examined, a sensory and physico-chemical analysis is done, also a shelf-life evalutation is conducted. All these results were collected and are discussed in this thesis.

2. Literature study

2.1. Nutritional value of *Hermetia illucens*

The actual nutrient content of the BSF depends highly on the given diet (Tschirner & Simon, 2015), on the life stage and the rearing conditions (Makkar, Tran et al., 2014). Because of this, different compositions of BSF are found in different studies.
St. Hilaire et al. (2007) found a DM content of 91.6% and ash content of 15.5% with the BSF prepupae.

2.1.1. Protein and amino acid

The maggot of the BSF contains 35-40% crude protein on DM base (Elwert et al., 2010).
Table 2 shows the different levels of amino acids in BSF larvae, fish meal and soybean meal. The difference in values between the two BSF larvae can be explained by the use of a different substrate for the larvae, since the actual nutrient content of the BSF depends highly on the growing substrate used as mentioned above.
Elwert et al. (2010) compared *Hermetia illucens* meal to fishmeal and found higher levels of threonine, valine, isoleucine, leucine and histidine in the insect meal relative to lysine. The methionine level was slightly lower. He concluded that BSF meal could serve as a source of protein for broilers since it has a similar high quality as fishmeal. Tryptophan was not determined. In table 1 is the amino acid composition of BSF larvae found by Al-Qazzaz (2016), which includes tryptophan.

Table 1: Amino acid composition of BSF larvae (Al-Qazzaz et al., 2016)

Amino acid	g kg^{-1}	Amino acid	g kg^{-1}
Arginine	93.31	Aspartic acid	48.08
Histidine	14.76	Alanine	52.45
Isoleucine	12.22	Cysteine	89.17
Leucine	37.33	Glutamic acid	65.81
Lysine	28.63	Glycine	10.9
Methionine	26.46	Proline	36.65
Phenylalanine	16.29	Serine	32.88
Threonine	22.36	Tyrosine	26.97
Valine	21.93		
Tryptophan	0.49		

Table 2: Content of amino acids (in % of crude protein) in BSF larvae, fish meal (Degussa, 1996) and soybean meal (Degussa, 1996)

Contents of essentials amino acids (in % of crude protein)				
	BSF larvae [1]	BSF larvae [2]	Fish meal [3]	Soybean meal [3]
Arginine	4.80	6.08	5.81	7.42
Histidine	3.28	2.71	2.82	2.77
Isoleucine	4.16	4.66	4.08	4.56
Leucine	6.56	7.11	7.20	7.81
Lysine	5.92	6.01	7.62	6.26
Methionine	1.60	1.70	2.81	1.45
Phenylalanine	3.60	4.59	3.99	5.26
Threonine	3.92	4.08	4.19	3.99
Valine	5.68	6.40	4.81	4.72
Contents of non-essential amino acids (in % of crude protein)				
	BSF larvae [1]	BSF larvae [2]	Fish meal [3]	Soybean meal [3]
Aspartic acid	8.16	8.53	9.31	11.86
Alanine	7.84	6.93	6.31	4.38
Cysteine	0.88	Not analysed	0.91	1.51
Glutamic acid	11.84	8.67	12.87	18.04
Glycine	5.60	5.23	6.82	4.38
Proline	6.24	5.48	4.43	5.18
Serine	4.32	3.85	3.96	5.18
Tyrosine	5.12	7.06	3.20	3.85

[1] Tschirner & Simon, 2015

[2] (St-Hilaire, Sheppard et al., 2007b)

2.1.2. Lipid and fatty acids

BSF has a crude fat content of 35% DM (Veldkamp et al., 2012). Like the amino acids, the composition of the fatty acids (FA) of the BSF depends strongly on the diet. St-Hilaire et al. (2007) found that BSF fed with cow manure had a different total fat content and a different FA profile than when fed with a mixture of cow manure and fish offal. By feeding the larvae fish offal, the lipid content of the BSF can be increased and manipulated to include desirable FA, such as α-linolenic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The omega-3 FA are enriched and a higher total FA content is found (St-Hilaire, Cranfill et al., 2007a). Omega-3 FA are long chain polyunsaturated fats and have many benefits for human health such as reduction of the risk of coronary heart disease

(Kralovec, Zhang et al., 2012), they are precursors to antiiflammatory mediators, prevent inflammatory mediated disorders such as allergy, diabetes, Alzheimer's disease and neurodegenerative diseases (Lavie, Milani et al., 2009).

The main lipid component of the BSF is lauric acid and its esters. Lauric acid is in animals and humans converted into monolaurin, which is an antiviral, antibacterial and antiprotozoal glyceride. Diglycerides of lauric acid and other FA are emulsifiers and stabilizers of disperse systems (Ushakova, Brodskii et al., 2016).
The BSF also contains azelaic and sebacic acid, whose esters may ensure the plasticity of the lipid complex of larvae at low temperatures. Insects are poikilothermic: they can survive in a wide range of temperatures because of their FA profile. The ability to survive in rotting vegetable substrates with temperatures above 40 °C, is because of the saturated lauric acid that prevents the rapid oxidation of fat. Azelaic acid also inhibits the reproduction of lipophilic microorganisms in skin sebaceous glands, this provides the protection of the lipid complex from infections (Ushakova et al., 2016).
Li et al. (2011) examined the lipid profile from the BSF fed with dairy manure. It revealed 10 kinds of FA predominantly from C:10 to C:22. 15.8% saturated and 39.8% unsatured fatty acids were found. Lauric acid (C12:0) was the highest saturated FA (35.6%) followed by palmitic acid (14.8%) (C16:0). The highest unsaturated fatty acid was oleic acid (23.6%) (C18:1n-9).
Also Surendra et al. (2016) found that lauric (C12:0), palmitic (C16:0) and oleic (C18:1) acids were the most dominating FA in the oil derived from BSF prepupae with relative percentages of 45, 14 and 12%. Respectively 10 and 8% linoleic (C16:2) and myristic (C14:0) acid were found.
Ushakova et al. (2016) studied the composition of the lipid fraction of the BSF fed on wheat grain. The 38.4 % of the total fatty acid methyl esters was lauric acid.
It is clear that the FA composition of the BSF is highly affected by the growing substrate, but the main lipid component remains lauric acid.

Most of the lipids are found under the form of triglycerides, synthesized from carbohydrates, fatty acids or proteins. Diglyceride esterizes in the presence of diacylglycerol acyltransferase to triglyceride. The BSF contains a higher amount of lipid than glycogen because of the higher capacity for lipid synthesis from carbohydrate than for glycogen synthesis (Arrese & Soulages, 2010).
The fatty acid profile of BSF prepupae oil and soybean oil can be found in table 3 (St-Hilaire et al., 2007b).

Table 2: Fatty acid composition of BSF prepupae raised on swine manure and FA composition of soybean oil (St. Hilaire et al., 2007) expressed on a weight percent of total lipid

Fatty acids	BSF prepupae oil	Soybean oil
Lauric (C12:0)	49.34	0.00
Myristic (C14:0)	6.83	0.00
Palmitic (C16:0)	10.48	11.30
Palmitoleic (C16:1n-7)	3.45	0.10
Stearic (C18:0)	2.78	3.60
Oleic (C18:1n-9)	11.81	24.90
Linoleic (C18:2n-6)	3.68	53.00
Linolenic (C18:3n-3)	0.08	6.10
Saturated Fatty Acids (SFA)	69.90	15.30
Monounsaturated Fatty Acids (MUFA)	14.90	25.60
Polyunsaturated Fatty Acids (PUFA)	12.50	59.10

2.1.3. Minerals

Tschirner & Simon (2015) examined the mineral contents of the BSF. These results can be found in table 4 beneath together with the mineral contents of the growing substrate and fish meal as comparison.

The calcium concentration of the BSF is lower than in fish meal, but the BSF has a higher calcium content than other insects (Jozefiak, Jozefiak et al., 2016).

Also the mineral content of the BSF depends highly on the given diet (Henry, Gasco et al., 2015) as shown in table 5. Pig manure resulted in the highest P-content of the BSF larvae (1.65% DM), followed by cow manure (1.27% DM). The N:P ratios of the larvae fed with cow, pig and chicken manure are respectively 5.44, 3.49 and 3.97.

Table 3: Mineral content of young larvae of BSF (Tschirner & Simon, 2015) and fish meal (NRC, 1994) (values per kg DM)

Mineral	Larvae	Fish meal
Ca (g)	22.26	24.62
P (g)	19.51	18.28
Mg (g)	5.73	1.61
K (g)	19.51	11.72
Na (g)	1.62	/
Mn (g)	0.25	5.38
Fe (g)	0.34	0.15
Zn (g)	0.23	0.14
Cu (mg)	23.6	6.45
Co (mg)	0.21	/
Mo (mg)	1.09	/
Cd (mg)	0.36	/
Pb (mg)	0.77	/

Table 4: Mineral content of dried black soldier fly prepupae raised on poultry and swine manure (Newton, 2005)

Mineral	BSF grown on poultry manure	BSF grown on swine manure
P (%)	1.51	0.88
K (%)	0.69	1.16
Ca (%)	5	5.36
Mg (%)	0.39	0.44
Mn (ppm)	246	348
Fe (ppm)	1370	776
B (ppm)	0	/
Zn (ppm)	108	271
Sr (ppm)	53	/
Na (ppm)	1325	1260
Cu (ppm)	6	26
Al (ppm)	97	/
Ba (ppm)	33	/

2.1.4. Chitin

Diener et al. (2009) found a chitin content in prepupae of the BSF of 8.72% DM.
Chitin is a widely occurring polysaccharide in nature and can be found in the cuticle of the BSF. It is usually found in a complex matrix with other compounds (Finke, 2007). Chitin is composed of β (1→4) linked 2-deoxy-2-acetamido-D-glucopyranosyl residues (Kramer, Hopkins et al., 1995).

Insects contain crude fiber (CF), acid detergent fiber (ADF) and neutral detergent fiber (NDF). ADF is composed of cellulose and NDF of cellulose, lignin and hemicellulose (Van Soest & Robertson, 1977). The components of the ADF and NDF in insects are unknown. Because chitin (linear polymer of β-(1→4) N-acetyl-D-glucosamine units) is similar to cellulose (linear polymer of β-(1→4)-D-glucopyranose units) and the ADF fraction contains nitrogen, chitin is believed to be the component of the fibers (Finke, 2007).
Chitin has an effect on the intestinal development: it reduces the nutrient digestibilty and has a prebiotic activity (Bovera, Loponte et al., 2016). Because chitin is indigestible, it affects the digestibility of protein in poultry. Poultry fed with a poorly digestible diet results in an increase of intestinal lenght and relative volume and weight (van der Klis and Jansman, 2002). This is a compensatory mechanism, the poultry increases the feed intake as well as the surface available for nutrient absorption (Borin et al., 2006). With diets with a low digestibility, the indigestible portion stays in the intestinal tract and can affect the growth perfomance of broilers because they can serve as a substrate for intestinal bacteria (Pieper, Jha et al., 2008). Another reason for the increase in intestinal length can be the ability of chitin to act as prebiotic (Bovera et al., 2016). Khempaka et al. (2011) showed that shrimp head meal (which contains chitin) as well as the addition of purified chitin increases the production of butyric volatile fatty acid in cecae in broiler chickens. Butyric acid is considered the prime enterocyte energy source (Mahdavi & Torki, 2009) and necessary for the development of the lymphoid tissue in the gut (Mroz, 2005). Butyrate can stimulate the growth of colorectal and ileal mucosal cells (Montagne, Pluske et al., 2003), which is important to maintain the function of the gastro-intestinal tract. With a higher amount of butyric acid, there is an increase of nutrients for enterocytes which enhances blood flow through the intestine which leads to tissue oxygenation and nutrient transport and absorption (Mahdavi & Torki, 2009). It is possible that this mechanism involves local neural networks and chemoreceptors that effects smooth muscle cells (Mroz, 2005). This may induce the production of growth factors that stimulate the growth of the intestine tracts (Mahdavi & Torki, 2009).
The intake of chitin has a bacteriostatic effect on Gram-negative bacteria, *E. Coli*, *Vibrio cholerae*, *Shigella dysenteria* and *Bacteroides fragilis* (Vidanarachchi, 2010) and a antifungal and antimicrobial effect (Khoushab & Yamabhai, 2010).

To increase the digestibilty of the insect meal, the chitin can be removed by multiple mechanisms. This can be done by alkali extraction (Defoliart, 1992), adding chitosan, chito-oligosaccharides or acetylglucosamine (Lin, Mao et al., 2012). Adding chitin degrading enzymes or bacteria can improve the digestibilty of the chitin-protein complexes (Kroeckel, Harjes et al., 2012).

2.1.5. Antimicrobal peptides

The BSF is, just as other insects, a rich source of antimicrobial peptides (AMP) or natural antibiotics, these are small cationic peptides. AMP cause damage to bacteria, fungi and certain parasites and viruses. By binding and interacting with lipids of the cell membranes, a change in cell membrane structure occurs. The interaction results in the incorporation of the AMP into the cytoplasmic membrane of the bacteria which affects the acid and protein synthesis. This may explain the effect on antibiotic-resistant microorganisms (Jozefiak et al., 2016).
Defensins are the largest group of insects AMP. The BSF produces a defensin-like peptide, its expression is induced in the fat body by bacterial challenge (Park, Kim et al., 2015).

2.1.6. Digestibility

De Marco et al. (2015) did a study on the digestibility of *Hermetia illucens* in broiler chickens. The apparent digestibility coefficients of the total tract (CCTAD) of different nutrients were found: 0.53 for DM; 0.66 for organic matter; 0.51 for crude protein; 0.99 for ether extract and 0.69 for gross energy. The CCTAD values for the different nutrients are not very high, except for the ether extract. It is possible that the chitin in the exoskeleton can negatively affect CCTAD of nutrients. Poultry can not digest chitin but it does not appear to have damaging effects on their performance (Ravindran & Blair, 1993). The apparent metabolizable energy was 17.38 MJ/kg DM and the nitrogen corrected apparent metabolizable energy was 16.60 MJ/kg DM. The apparent metabolizable energy of soybean meal is comparable with a value of 16.93 MJ/kg DM (Sotak-Peper, Gonzalez-Vega et al., 2015). The apparent ileal digestibility coefficients (AIDC) can be found in table 6. The overall apparent ileal digestibility coefficient was 0.68. These values are lower than those for soybean meal, where the overall apparent ileal digestibility coefficient was 0.87, on average (Huang, Li et al., 2006).

Table 5: The AIDC of amino acids of BSF (De Marco et al., 2015) and soybean meal (Huang et al., 2006)

AIDC					
Indispensable amino acids	BSF	Soybean meal	Dispensable amino acids	BSF	Soybean meal
Arginine	0.83	0.91	Alanine	0.86	0.86
Histidine	0.81	0.88	Aspartic acid	0.61	0.86
Isoleucine	0.45	0.87	Cysteine	0.82	/
Leucine	0.76	0.86	Glycine	0.67	0.84
Lysine	0.46	0.90	Glutamic acid	0.74	0.89
Methionine	0.42	0.93	Proline	0.89	/
Phenylalanine	0.63	0.87	Serine	0.82	0.85
Threonine	0.75	0.81	Thyrosine	0.43	0.88
Valine	0.62	0.86	Mean	0.73	0.86
Mean	0.64	0.88			

2.2. Production of black soldier fly

Insect-based feed products can replace conventional feed ingredients with a high environmental impact. By using the insect-based feed, the environmental impact of livestock production may be reduced (van Zanten, Mollenhorst et al., 2015). The BSF can be grown on organic side streams hereby reducing the environmental contamination, the waste is being transformed into high protein feed (Sanchez-Muros, Barroso et al., 2014). One third of all food is being wasted, which is about 1.3 billion ton per year (Gustavsson et al., 2011). This wasted food can be used as a feed source for insect production. Not only food waste can be reduced by the BSF, they can also grow on manure (van Huis, 2013). Larvae fed with dairy manure can reduce the DM mass by 58%. Hereby, P is 61-70% reduced and N 30-50% (Myers et al., 2008). Newton et al. (2005) measured a reduction of 56% DM of fresh swine manure and Sheppard et al. (1994) found a 50% reduction of hen manure. The reduction of minerals found by Newton (2005) is shown in table 7. Due to high larval denisities, the organic waste is processed extremely fast and bacterial growth is suppressed. Because of this, the bad odour production is kept to a minimum (Diener et al., 2011). This reduction of manure can reduce the pollution potential with 50-60% or more (Newton et al, 2005). Insect production does not require additional drinking water. Insects use water very effectively and use the feed as water source (Jozefiak et al., 2016).

The most suitable feeding rate to efficiently reduce waste is 100 mg of food per larvae per day (Diener, Zurbrugg et al., 2009), but larvae fed with 200 mg chicken feed had the highest prepupal weight (63.3 mg) and a shorter development time (15.9 days) than larvae fed with 100 mg per day (48.0 mg and 16.6 days) (Diener et al., 2009).

The larvae growth and maturity which correlates with the size of the adult flies and their survival is influenced by the organic waste quality. Different types of organic waste will result in different development times (Leong, Kutty et al., 2016). The shortest development time is found when the larvae are fed with kitchen waste or liver. Next in line are diets with fish and vegetables. Then comes chicken and pig manure, and larvae fed with cow manure have the longest development time (Oonincx et al., 2015).
The lowest mean weight and length of the larvae is found with BSF fed with manure while the highest mean weight and length result from poultry feed, kitchen waste and liver. The low energy, protein and fat content per unit of dry matter in fruit, vegetables and manure explain the poor performance of the larvae (Nguyen, Tomberlin et al., 2013).
In case of food shortage, larvae will feed until they have reached the minimum energy reserve required to perform pupal development. The larvae will only migrate as soon as they reach ±35 mg DM, the critical weight (Diener et al., 2009). The prothoracicotrop hormone (PTTH) causes the larvae to stop feeding, there is a gap between the moment of achieving the critical weight and the secretion of PTTH. The weight gain in this period can explain the differences in prepupal weight between the various feeding types (Diener et al., 2009).

Table 6: Concentration of selected elements (DM) in pig manure and the concentration of the residue after being digested by the BSF (Newton et al., 2005)

Element	Pig manure (ppm)	Black soldier fly residue (ppm)	Change (%)
N	923.7	414.52	-55.1
P	676.2	378	-44.1
K	358.7	169.34	-52.8
Ca	969.3	425	-56.2
Mg	299.3	175.96	-41.2
S	80.31	44.44	-44.7
Fe	6.63	6.8	+2.6
Mn	12.8	6.02	-53.0
Zn	23.53	12.91	-45.1
Cu	14.85	8.05	-45.8
B	0.32	0.16	-50.0
C	11,248	4,232.6	-62.4
Na	99.93	48.15	-51.8

For the optimal production of the BSF, there has to be good knowledge on housing conditions and feed. Knowledge about insect diseases and biosecurity standards for the production is essential. Good housing conditions are necessary for a good production as the growth rate and feed utilization highly depend on temperature (Jozefiak et al., 2016). The needed temperature is a disadvantage in the production since it can be energy-consuming (Makkar et al., 2014). Light intensity influences the number of BSF mating, a light intensity less than 63 µmol $m^{-2}s^{-1}$ results in no mating.
Not only the light intensity influences this process, also the wavelenghts. Absence of certain wavelenghts can obstruct the mating. Mating occurs more frequently early in the day, and as the day progresses, it decreases. In contrast, more eggs are deposited later in the day. Temperature and humidity are positively regressed with egg oviposition. With a humidity above 60%, 80% of egg clutches are deposited (Tomberlin & Sheppard, 2002).
The BSF is not a disease vector: the adult fly can not eat so does not come in contact with unsanitary wastes. Also, the eggs are never laid on decaying organic material (Ryan, 2014). Additionally, the larvae can modify the microflora of manure, hereby the harmful bacteria can be reduced such as *Escherichia coli* and *Salmonella enterica* (Erickson, Islam et al., 2004). Another important aspect is that they eliminate housefly breeding (Sheppard et al., 1994).

The prepupae can be self-harvested because of the migration of the larvae. To pupate to an adult, the larvae need to leave the manure they are grown on. At that moment, they are at their maximum size and have a large storage of fat. The migrating prepupae no longed feed themselves, they use their mouthparts to pull their body along (Newton et al., 2005). Newton et al., (2005) used a larval culture basin wiht 90,000 to 100,000 mixed aged larvae/m^2. Along the opposing walls of the basin, a 35° ramp led the prepupae to a gutter at the top, which led them to collection in containers. A part of the prepupae was used for the animal feed, the other part was used to sustain the adult soldier fly colony which eggs were used to maintain the larval denisity needed to digest the manure.

2.3. The use of *Hermetia illucens* in feed

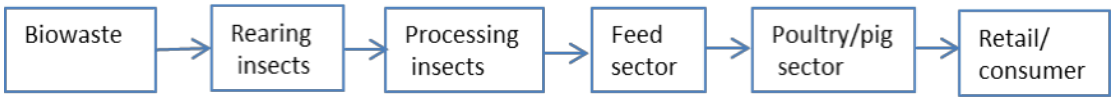

Figure 1: Insects in the feed chain (Veldkamp, 2012)

Mostly soy bean meal and fishmeal are used in poultry feed, because of the high economical and environmental cost of these ingredients, an alternative needs to be found.
Insects are a promising feed ingredient because of multiple reasons: they contain a high quantity

and quality protein, they can be easily produced and handled and have short life cycles. On top of that they can recycle food waste materials (Ramos-Elorduy, Gonzalez et al., 2002). Figure 1 displays a possible process chain of insects used as feed.

In the Catalogue of Feed Materials (EC68/2013) there is a listing for 'whole or parts of terrestrial invertebrates' but nothing for 'insect meal'. Insects that are used in meal must meet the requirements of Directive EC 2002/32 on Undesirable Substances in Animal Feed, this gives the maximum permitted levels of contaminants.
Insects have to be progressed according to the EU Animal By-Products Regulation 1069/2009 to become processed animal protein (PAP) so they can be fed to animals. Non-pathogenic insects are classified as category 3, low risk material, so they should be suitable for animal feed. But because of the BSE outbreak, all PAP is forbidden being used in animal feed, with the exception of hydrolysed proteins. There is an exception for fishmeal that enables the use of non-ruminant PAP in farmed fish species intended for human consumption. Because this regulation concerns processing in slaughterhouses and controls, insects PAP is not allowed because they don't follow the same route as livestock (Koeleman, 2015).

In the near future there is a change in this regulation expected, that includes the admission of insects in pig and poultry feed (Jozefiak et al., 2016).

Table 8 contains a summary of experiments where BSF was fed to poultry and the effect on the animal perfomances.

Table 7: Experiments done on poultry with BSF as feed

Elwert et al. (2010)	Broiler chickens fed with non-de-fatted BSF meal have a similar body weight after 10 days as compared to fishmeal diet. Increasing level of de-fatting the BSF meal resulted in a decreasing feed conversion and body weight. There was hardly and effect on the feed conversion rate. Feed conversion was lower in the grower period with Hermetia meal in comparison to an all-vegetable diet, but there was a lower percentage of losses. Conclusion: BSF meal can serve as a source of protein for broilers.
Cullere et al. (2016)	Broilers quails fed with a control diet, diet with 10% BSF larvae and a diet with 15% BSF larvae showed the same slaughter weight, body weight gain, feed intake, feed conversion ratio and mortality rate. Also dry matter intake and excreta production were the same for the 3 diets. Apparent digestibility of nutrients was only for EE higher in the control group and the group with 15% BSF larvae.
(Al-Qazzaz, Ismail et al., 2016)	Laying hens were fed with a control diet (T1), 5% BSF larvae inclusion diet (T2) and a 1% BSF larvae inclusion diet (T3). T2 showed the highest daily egg production. The egg weight of T2 was significantly lower than the egg weight of T3. Feed conversion ratio was significantly higher with inclusion of BSF larvae.
Widjastuti et al. (2014)	Quails were fed with 5 different diets: 0% inclusion BSF (R0), 2.5% inclusion BSF (R1), 5% inclusion BSF (R2), 7.5% inclusion BSF (R3) and 10% inclusion BSF (R4). Higher levels of BSF replaced fish meal. Feed consumption reduced with an increasing level of BSF in the diets. There was no effect found on the egg production. Feed conversion ratio is better with quails fed with BSF than without BSF in the diet. R2 gave the best result of feed conversion ratio. Conclusion: substitution of fish meal by BSF maggot meal until 5% level can support a good result on quail production performance.

2.4. Laying quails farming

Quails can be housed in outdoor aviaries, floor pens or battery-type caging. In outdoor housing the quails must have access to shelter and there can be an increased risk of disease. The battery cage is frequently used in farming and research. These cages are small, don't allow exercise of the quails and don't provide environmental stimulation (Hawkins, Morton et al., 2001). Quails can be sexed from three weeks old based on their feather colour or pattern (Hawkins et al., 2001). They are generally provided with a starter diet until they are 21 days old, after that they get a grower diet until six weeks old and then a breeder diet.

Quails mature in five to six weeks and the lighting has a great influence on the duration of this process (Woodard et al., 1973). Also the production is affected by the lighting, Coban (2008) used in his experiment a photoperiod of 14 hours and 10 minutes. Woodard (1973) found a photoperiod of 14-18 a day for optimal production. When continuous lighting is used, there is a lower production and a higher daily consumption. Also the viability is lower, but no significant egg weight and body weight differences were found (Ionita, Popescu-Miclosanu et al., 2015).

Quails lay eggs on three out of four days on average (Hawkins et al., 2001). When hens are 26 weeks old, the rate of laying eggs decreases sharply (Woodard & Abplanalp, 1971).

The thermal comfort range of mature quails is from 18 to 22°C, and the relative humidity is optimal from 65 to 70% (Oliveira cited in Rodrigues et al., 2004).

2.5. Egg quality

Several factors need to be considered to discuss the egg quality. The egg quality can be affected by age, breed, origin, care, nutrition, temperature, diseases, etc.. The period of storage and the temperature of the eggs can effect the interal quality (Sari, Tilki et al., 2016). The external parameters to judge the egg quality are egg weight, egg shape, shell weight and thickness (Altan, Oguz et al., 1998). To determine the internal quality the following factors have to be considered: albumen length, albumen width, albumen height, yolk width, yolk height (Sari et al., 2016) and yolk color score (Tufarelli, Cazzato et al., 2016). Zita et al. (2013) reported a yolk colour score of 4.87.

Sari et al. (2016) found the following results for the internal and external quality parameters:

Table 9: Internal and external egg quality parameters (Sari et al., 2016)

External factor	Value	Internal factor	Value
Egg weight (g)	11.90	Yolk weight (g)	3.90
Egg length (cm)	3.30	Yolk diameter (cm)	2.40
Egg width (cm)	2.60	Yolk height (cm)	1.10
Shape index (%)	78.90	Yolk index (%)	44.20
Shell weight (g)	1.30	Yolk ratio (%)	33.10
Shell thickness (mm)	0.20	Albumen weight (g)	6.70
Shell ratio (%)	10.80	Albumen height (mm)	5.90
		Albumen ratio (%)	56.10
		Haugh unit	96.20

2.5.1. Chemical composition

Dudosola (2010) did research for the proximate composition of the egg, seperatly for the whole egg, yolk and albumen. The results are in table 10. The found values are similar to the found values of other authors (Imai, Mowlah et al., 1986, Song, Choi et al., 2000).

Table 10: Chemical composition of quail eggs (Dudusola, 2010)

	Whole egg	Yolk	Albumen
Moisture (%)	74.26	49.71	87.82
Crude protein (%)	11.98	15.99	10.39
Crude fat (%)	11.91	31.48	0.09
Crude ash (%)	1.04	1.79	1.00

Polat researched the fatty acid composition of yolk in nine poultry species kept in their natural environment. The results for the yolk of the quail egg can be found in table 11. The main fatty acids are oleic acid (C18:1), palmitic acid (C16:0), linoleic acid (C18:2) and stearic acid (C18:0). Comparable results were found by Roll et al. (2016).

Table 11: Fatty acid composition of egg yolk from quail (Polat, Citil et al., 2013)

Fatty acid	%	Fatty acid	%	Fatty acid	%
C10:0	0.01	C14:1	0.18	C18:2	22.17
C11:0	0.01	C16:1	4.65	C18:3	0.44
C12:0	0.02	C18:1	39.91	C20:4	1.93
C13:0	0.02	C20:1	0.25	C20:5	0.08
C14:0	0.77	C22:1	0.03	C22:2	0.00
C15:0	0.21	MUFA	45.02	C22:3	0.07
C16:0	22.77			C22:4	0.01
C17:0	0.23			C22:5	0.16
C18:0	5.53			C22:6	0.22
C20:0	0.09			PUFA	25.09
C21:0	0.12				
C22:0	0.12				
SFA	29.9				

Genchev (2012) conducted an experiment where he compared the quality traits and the composition in quail eggs between the Pharaoh breed and the Manchurian Golden breed. The found amino acid profile is in table 12. The mineral composition of the yolk, albumen and egg shell is showed in table 13.

Table 12: Amino acid profile of quail eggs (yolk and albumen) for Pharoah breed and Manchurian Golden breed (MG) (Genchev, 2012)

Essential amino acid	Pharoah (%)	MG (%)	Non-essential amino acid	Pharoah (%)	MG (%)
Threonine	0.73	0.73	Aspartic acid	1.3	1.29
Valine	0.9	0.89	Serine	0.94	0.92
Methionine	0.44	0.43	Glutamic acid	2.06	2.02
Isoleucine	0.65	0.65	Proline	0.64	0.64
Leucine	1.24	1.23	Cysteine	0.57	0.55
Phenylalanine	0.78	0.76	Glycine	0.44	0.44
Histidine	0.48	0.48	Alanine	0.72	0.72
Lysine	1.18	1.17	Thyrosine	0.52	0.51
Arginine	0.49	0.48			
Σ essential AA	7.01	6.92	Σ non-essential AA	7.07	6.99

Table 13: Mineral content of yolk, albumen and shell of quail eggs (Genchev, 2012)

	Yolk	Albumen	Shell
Ca (mg/kg)	1490.00	85.00	309200.00
P (mg/kg)	4880.00	100.00	3000.00
Mg (mg/kg)	111.41	83.25	84.62
Fe (mg/kg)	39.39	2.07	27.12
Cu (mg/kg)	0.62	0.45	2.60
Zn (mg/kg)	18.98	1.37	6.43

2.5.2. Correlation between egg quality parameters

A correlation was found between shell weight and yolk weight (r = 0.470), shell weight and albumen weight (r = 0.539), yolk weight and albumen weight (r = 0.654), shell weight and egg weight (r = 0.654), yolk weight and egg weight (r = 0.77) and albumen weight and egg weight (r = 0.932) (Orhan, Eyduran et al., 2016).

The egg shape has an effect on the hatchability of the eggs and on the desirability of the commercial eggs. Round eggs, with a higher egg shape index have a lower hatchability (Sari et al., 2016).

The egg shell functions as protection of the egg content from mechanical impact and microbal invasion, it also controls water and gas exchange through the pores during the extra-uterine development (Narinc, Aygun et al., 2015). The weight loss of the egg, embryonic mortality, hatchability and early chick growth rates are influenced by the egg shell quality (Roberts, 2004). Eggshell thickness decreases as the quails get older (Genchev, 2012). The eggshell thickness can vary within 130-280 μm, depending of the quail breed. The highest values are found during the first productive month of the quails (Genchev, 2012). Eggshell thickness is also influenced by the cholesterol content of the yolk. Eggs with a higher cholesterol content have lighter and thinner eggshells (Panda, Reddy et al., 2003). Table 15 shows the Pearson correlation coefficients found between different egg parameters found by Alkan et al. (2010)

2.5.3. Nutrients that affect the egg quality

Arginine and lysine play an important role regarding to the development of growth, egg production and egg quality. Deficiencies of the amino acids lead to a lower body weight gain, lower feed intake and a lower feed conversion ratio. On the other hand can excessive levels of arginine en lysine lead to an impaired growth performance and no positive effects are found on the egg production and quality (Bulbul, Ulutas et al., 2015). There is no impact on the feed intake, egg production, or weight and quality with increasing arginine:lysine ratios if both are present in adequate levels (Reis, Barreto et al., 2012).
An optimal balans of Na, K and Cl ensures a good egg productivity and egg quality. Na and H_2CO_3 play an important role in the egg productivity and shell formation. Ca is the most important restrictive factor of the shell formation (Mongin, 1968). The acid-base balance in the blood affects the eggshell formation, because this balance can be a restricting factor for the accumulation of $CaCO_3$, which is the main component of the eggshell (Ozbey et al., 2004). The balance can be negatively affected by high-temperature stress (Odom, 1989), so a thinner egg shell is formed when the quail has high-temperature stress. Also a lower egg weight is obtained with high temperatures (Ozbey et al., 2004).
The interaction between Ca en available P (avP) influences the egg quality. A combination of 38 g Ca/kg feed and 3.0 g avP/kg feed results in the highest egg weight. With a amount of 38 g Ca/kg the heighest eggshell weight and eggshell percentage was obtained, and the albumen percentage decreased. This last effect is a consequence of the Ca binding to proteins which reduces the availability of proteins to sythesize the egg albumen (Ribeiro, Barreto et al., 2016).

The yolk color depends on the concentration, type and ratio of xanthophylls in the feed. By adaption of the feed ingredients, the color can be changed. Also by adding synthetic and/or natural pigments (Galobart, Sala et al., 2004).

The cholesterol content can be affected by the level of MUFA in the diet. A higher level of oleic acid is associated with a decreasing level of total cholesterol and low density lipoprotein cholesterol (LDL-C) and an increasing level of high density lipoprotein cholesterol (HDL-C), which is beneficial for human health (Roll et al., 2016). Roll (2016) found that replacing soybean oil with canola oil resulted in different concentrations of the MUFA, oleic acid and the PUFA, linoleic acid in the yolk.

2.5.4. Genitical influences on the egg quality

Alkan et al. (2010) studied the effect of different lines of Japanese quails on internal and external quality traits of the eggs. He used four different lines: high body weight line (HL), low body weight line (LL), random bred control line (CL) and a layer line (L). The mean values of this experiment are given in table 14. This shows that genetic factors have a big effect on egg weight, egg length, egg width, yolk index values, egg shape index, eggshell weight and eggshell thickness.

Quails with a higher body weight, will have heavier eggs (Alkan et al., 2010, Ozdemir & Inci, 2012). Also the shape index, albumen weight, yolk weight, shell thickness and shell weight increases as the body weight of the quails are higher (Ozdemir & Inci, 2012).

Table 14: Means of egg quality parameters in four different quail lines (Alkan et al., 2010)

Traits	HL	LL	CL	L
Egg weight (g)	14.14	9.23	10.49	11.43
Egg width (mm)	27.13	23.56	24.93	25.65
Egg length (mm)	34.71	30.02	31.24	33.41
Yolk height (mm)	12.03	10.51	10.71	11.09
Yolk width (mm)	27.01	21.99	24.41	25.43
Albumen length (mm)	51.15	40.80	48.07	49.50
Albumen width (mm)	38.92	31.54	37.49	38.56
Albumen height (mm)	4.58	3.90	3.78	4.10
Shell thickness (mm)	0.231	0.226	0.225	0.234
Shell weight (g)	1.15	0.84	0.91	1.00
Haugh unit	87.96	88.28	86.07	87.01
Egg shape index (%)	78.23	78.62	79.88	76.8
Yolk index (%)	44.57	47.33	43.28	43.67
Albumen index (%)	10.08	10.67	9.40	9.33

Table 15: Pearson's correlation coefficients among the egg quality traits (Alkan et al., 2010)

Traits	EWi	EL	YH	YW	AH	AW	AL	SW	SI	ST	HU	YI	AI
EWe	0.938[a]	0.889[a]	0.727[a]	0.870[a]	0.402[a]	0.548[a]	0.636[a]	0.752[a]	-0.170[a]	0.132[b]	-0.018	-0.216[a]	-0.072
EWi		0.821[a]	0.676[a]	0.877a	0.368[a]	0.567[a]	0.657[a]	0.698[a]	0.044	0.149[b]	-0.038	-0.270[a]	-0.101
EL			0.597[a]	0.806[a]	0.339[a]	0.533	0.641[a]	0.734[a]	-0.531[a]	0.219[a]	-0.072	-0.242[a]	-0.127[b]
YH				0.552[a]	0.498[a]	0.271[a]	0.369[a]	0.530[a]	-0.047	0.049	0.207[a]	0.341[a]	0.162[b]
YW					0.239[a]	0.653[a]	0.686	0.661[a]	-0.114	0.124	-0.163[b]	-0.507[a]	-0.228[a]
AH						-0.065	0.0044	0.270a	-0.048	-0.077	0.813[a]	0.234[a]	0.672[a]
AW							0.763[a]	0.393[a]	-0.094	0.103	-0.366[a]	-0.456[a]	-0.523[a]
AL								0.432[a]	-0.148[b]	0.063	-0.248[a]	-0.382[a]	-0.460[a]
SW									-0.240[a]	0.347[a]	-0.076	-0.127[b]	-0.068
SI										-0.156[b]	0.073	0.027	0.067
ST											-0.159[b]	-0.020	-0.080
HU												0.392[a]	0.766[a]
YI													0.422[a]

EWe: Egg weight, EWi: Egg width, EL: Egg length, YH: Yolk height, YW: Yolk width, AH: Albumen height, AW: Albumen width, AL: Albumen length, SW: Shell weight, SI: Shape index, ST: Shell thickness, HU: Haugh unit, YI: Yolk index, AI: Albumen index

[a] $P<0.01$ [b] $P<0.05$

3. Materials and methods

The experiment is conducted on a farm with laying quails in the Veneto region, Italy. The experimental design consists of three treatments of different diets: a control diet, a diet with 10% *Hermetia illucens* (H1) and a diet with 15% *Hermetia illucens* (H2). The diets are formulated so the protein level is equal in all three. The insect meal composed of BSF prepupae is bought at PROTIX company, the chemical composition can be found in table 16. The ingredients of the diets can be found in table 17 and the chemical composition in table 18.

Each diet has 5 replicates with each 15 quails, per treatment there are 75 quails and a total of 225 quails. The quails are three months old.

The quails are kept in battery cages with a constant access to water and feed. There is light 24 hours a day.

The ranging of the battery cages in showed in figure 2.

7A H2	1A C	1B C
8A C	2A H1	2B H1
9A H1	3A H2	3B H2
	4A C	4B C
	5A H1	5B H1
	6A H2	6B H2

Figure 2: Ranging of the battery cages with the cagenumber and treatment

Table 16: Chemical compostion of BSF meal

Component	Amount
DM (%)	94.61
Ash (%)	7.27
CP (%)	51.81
EE (%)	14.78
Ca (%)	0.977
Phosphorus (%)	0.833
Metabolisable nergy (kcal/kg)	3692

Table 17: Ingedrients of the different experimental diets

Ingredient (%)	C	H1	H2
Maize	48.26	52.39	54.58
Wheat flour	3.00	4.96	5.43
Soybean flour	35.91	22.96	16.60
Insect flour HI	0.00	10.00	15.00
Calcium carbonate	7.05	6.80	6.80
Dicalcium phospate	0.15	0.00	0.00
Salt	0.26	0.27	0.27
Methionine	0.24	0.24	0.24
Lysine	0.03	0.03	0.03
Vitamin-mineral premix	0.50	0.50	0.50
Soybean oil	4.60	1.85	0.55
Total	100	100	100

Table 18: Chemical composition of the different experimental diets

	C	H1	H2
Energy (MJ/kg)	16.431	16.527	15.831
Calcium (ppm)	38834.1	31989.2	33608.3
Phosphorus (ppm)	3391.8	3492.9	3669.4
Starch(%)	31.6	35.1	38.2
DM (%)	90.6	90.8	90.9
CP (%)	21.4	22.0	21.5
EE (%)	7.7	5.8	5.9
ASH (%)	12.4	11.8	11.5

At the beginning of the experiment, all quails are weighed. The first three weeks are an adaption period; in this period the eggs are counted and weighed every day. The fourth week all eggs are collected and marked daily. The eggs from the collection period are analysed. After the collection period an extra 20 eggs per treatment are collected to measure the shelf-life. Three kinds of analysis are conducted on the eggs: sensory, physico-chemical and shelf-life analysis.

3.1. Sensory analysis

The sensory analysis is conducted on 60 eggs/treatment. These eggs are transported to the Sensory Analysis Devision of the "Istituto per la Qualità e le Tecnologie Agroalimentari, Laboratorio Analisi Sensoriale (Veneto Agricoltura)". A descriptive sensory analysis is done by a trained panel to detect possible differences between the dietary treatments. A score is given on the following factors: scent, taste, sweetness and saltyness of the yolk. These factors are also measured on the albumen. These scores can go from 1 untill 9, where 1 represents the lowest/worst value and 9 the highest/best value.

3.2. Physical analysis

On 225 eggs/treatment is a physical analysis done. These eggs are transported to the Meat Science laboratory of the MAPS Department of the University of Padova. The egg weight and highest and lowest diameter are measured with a digital calibrator with an accuracy of ±0.1 mm. The egg shape was calculated by dividing the width by the height of the egg and multiply by 100. The surface area was calculated with the formula of Carter (1975) cited by Anderson (2004): surface area = 3.9782 * egg weight$^{0.7056}$.

After measuring each egg, they are broken, the egg shell is dried with a paper towel and weighed (±0.1 g). The thickness of the shell is measured with a digital calibrator with an accuracy of ±0.01 mm. The pH of the albumen (±0.1) and the colour value of the yolk (scale of Roche) is determined. The Roche Yolk Colour Fan is a standard for measing the yolk colour, it is widely accepted throughout the food chain. Each fan blade represents a colour than can be seen in the egg yolk, this method can objectively measure colour. The higher the Roche number is, the darker and more orange.

3.3. Chemical analysis

The contents of 7 eggs are homogenized to 1 sample which is put in a marked aluminium scale, weighed and stored at -40°C and then freeze-dried, afterwards these are weighed again and grinded. These samples are used for NIRS reading. From the 12 worst from NIRS estimations per treatment a chemical analysis is done: proximate composition (DM, crude protein (CP), ether extract (EE), ash), cholesterol content and FA profile.

DM is measured by weighing 3 ± 1 gram of each sample and putting this in the oven (105°C) for 24 hours. These samples are weighed again and put in the muffle furnace at 400°C for 24 hours after burning the samples until all the smoke dissapeared. The ash can be calculated by the weight of the sample that is left.

Crude protein is measured with the Kjeldahl method. 0.5 grams of each sample is put in a Kjedahl tube, hydrochloric acid is added which converts the nitrogen to ammonium ions. The tubes are put in a heat bath and the ions convert to ammonia gas. After the tubes are cooled down, the sample has to be distilled, the ammonia gas is led into a trapping solution where it turns into ammonium ions again. By titration with a standard solution, the ammonia can be calculated. With formulas, the amount of nitrogen can be determined. By multiplying this number to 6.25, the crude protein content is calculated.

To find the fatty acid profile the samples first have to be extracted, this extraction happens through a machine, the Dionex Ase 350. 1 gram of the sample and 2 grams of hydromatrix are mixed and put in a cell. These cells go in the machine, as well as an empty vial for each sample. In this vial the fat is collected. When the process is finished the vials are weighed to determine the extracted fat quantity. At this point the esterification begins, in every vial 1.5 ml of a 4% H_2SO_4 is added. After putting it in an oven of 60°C for 30 minutes, the samples are vortexed. The content of the vials are transferred to tubes of 4 ml, these are put again in the oven for 2 hours and are vortexed every half hour. The tubes are left overnight in the oven, and after vortexing them the next morning they are put again in the oven for a half hour. 0.5 ml of distilled water and 1.5 ml of n-heptanol is added, the samples are vortexed again. After centrifuging al the samples, 700 µl of the upper layer is transferred in a tube of 2 ml. On these tubes gas chromatography is conducted.

3.4. Shelf-life analysis

The shelf-life analysis is conducted on 16 eggs/treatment. On day one, the thiobarbituric acid reactive substances (TBARS) are measured on 8 eggs/treatment. On day 28 this is measured again on the rest of the eggs. The method of Botsoglu et al. (1994) is used to measure the concentration of malonaldehyde (MDA) which is an indicator for oxidation. 8 ml of a trichloroacetic acid (TCA) 5% solution and 5 ml of hexane and butylhydroxytolueen (BHT) (0.002 g BHT/100 ml hexane) is added to 2 grams of the sample. After homogenizing with Ultra Turrax, the samples are centrifuged for 3 min, the top hexane layer is discarded. The samples are filtrated and 2.5 ml of 2-thiobarbituric acid solution (TBA) (0.02 M) is added to 2.5 ml of the filtrate and put at a 100°C for 35 minutes. After the samples are cooled down,

spectophotometry is conducted at 532 nm. The blanc consists of 2.5 ml TCA 5% and 2.5 ml TBA. We can get the amount of mg MDA/kg egg out for every sample out of a curve.

3.5. Live performances

The performance of the layers is measured by registering the following factors: initial and final live weight, number of eggs, egg weight and mortality. These factors are calculated on a cage base. At the beginning and at the end of the trial all the quails are weighed, every days the layed eggs per cage are counted and noted. Each egg is weighed with an accuracy of ±1 g. The weight of dead quails are noted.

3.6. Statistics

All the data from the experiment is stastically processed with SPSS 24.0 software. All variables were tested for normality with the Kolmogorov-Smirnov test. A significance level of 5% was used. During the processing, the different diets were compared.
If a parameter was normal a Levene's test was used to test the equality of variances. The normally distributed parameters with equals variances were subjected to a one-way anova. If the zero hypothesis was rejected (the mean of the populations are not equal), a Tuckey-test was done on the variable to find in which groups the differences in means were.
Non-normally distributed parameters were tested with a Kruskal Wallis test.

4. Results

4.1. Sensory analysis

As is shown in table 19, the sensory quality on all aspects does not differ significantly between treatments (P>0.05). It is shown that palmitic acid, stearic acid and oleic acid have a neutral taste (Ledahudec & Pokorny, 1991), which are the most dominant fatty acids in the quail eggs. The fatty acid profile of the eggs in different treatments do not differ a lot, no difference in taste between the treatments was expected.

Table 18: The mean values, standard (STD) errors and P-values of the different parameters of the sensory analysis

	Diet	Mean	STD-error	P-value
Smell yolk	C	5.87	0.068	0.398
	H1	5.80	0.84	
	H2	5.87	0.73	
Bitterness yolk	C	6.09	0.79	0.622
	H1	6.10	0.87	
	H2	6.04	0.085	
Sweetness yolk	C	3.54	0.088	0.622
	H1	3.44	0.077	
	H2	3.43	0.10	
Saltiness yolk	C	3.69	0.086	0.076
	H1	3.53	0.069	
	H2	3.51	0.068	
Stickiness yolk	C	6.19	0.11	0.412
	H1	6.02	0.11	
	H2	6.04	0.081	
Smell albumen	C	5.38	0.066	0.728
	H1	5.37	0.77	
	H2	5.39	0.069	
Saltiness albumen	C	3.00	0.089	0.417
	H1	2.91	0.090	
	H2	2.96	0.091	
Bitterness albumen	C	5.30	0.073	0.806
	H1	5.35	0.079	
	H2	5.33	0.069	

Consistency	C	5.69	0.13	0.645
albumen	H1	5.63	0.11	
	H2	5.54	0.10	
Taste	C	6.85	0.15	0.588
	H1	6.72	0.14	
	H2	6.87	0.15	

4.2. Physical traits of the eggs

The different diets don't have an influence on the egg weight, the surface area and the albumen ($P>0.05$). Table 20 shows the different physical traits of the eggs in the different diets.

The egg weight found in the literature study (11.9 g) is lower than found in the experiment. In both researches, the quails were 3 months old, the difference can be explained by different quail breeds, nutrition and environmental conditions. Sari (2016) used a feed with a lower content in energy and lower amount of lighting per day. This can explain a lower intake of energy, so lower egg weight. The quails in the research of Sari (2016) had the same age as the quails used in this experiment but the weight of the quails is unknown. It is possible that the quails weighed less than in this experiment and as a consequence lay lighter eggs.
There is no significant difference of egg weight between the 3 treatments ($P>0.05$), this is because the quails have the same weight. If some quails were heavier, they would lay heavier eggs (Alkan et al., 2010).

Also no significant difference is found of the surface area between the treatments ($P>0.05$), this can be explained by a strong correlation with the egg weight.

The egg shape of C (mean: 75.20%) is significantly lower than the shapes of H1 (mean: 76.27%) and H2 (76.08%). The width of the C group (mean: 26.92mm) is significantly smaller than the width of H2 (mean: 27.20 mm). This means that the control group has a significantly smaller width (mean: 26.93 mm) than H1 (mean: 27.20 mm) and H2 (mean: 27.11) ($P<0.05$). Also a bigger height is found with the control group, but this difference is not significant ($P>0.05$).

The shell weight of C (mean: 1.44 g) is significantly lower than those of H1 (mean: 1.52 g) and H2 (mean: 1.52 g). As egg shape and shell weight are negatively correlated and the shell thickness is significantly higher in the C group, a significantly higher shell weight was expected

in C. The egg shell percentage obtained from the control diet (mean: 10.12%) is significantly lower than the percentages of H1 (mean: 10.43%) and H2 (mean:10.52%) ($P<0.05$). As the egg shell portion and egg edible portion percentage depend on one another, the egg edible portion of the C is significantly higher than H1 and H2 ($P<0.05$). The shell percentage of C is lower because the shell weight of C is significantly lower compared to H1 and H2, but the total weight of the eggs are equal.

The egg shell thickness of C (mean: 0.2140 mm) is significantly higher than the egg shell thickness of H1 (mean: 0.2104 mm) and H2 (mean: 0.2136 mm). This is because the amount of Ca in the C diet is higher than the diets of H1 and H2. Ribeire et al. (2016) showed that the maximum egg shell thickness is expected with an amount of 38 g Ca/kg in the feed. The C diet has 39 g Ca/kg while H1 and H2 have ony 32 and 34 g Ca/kg respectively. A thicker egg shell in the C group was expected.

The C group (mean: 4.99) gives a significantly lower Roches value for the yolk color than the H1 (5.57) and H2 (mean: 5.81) group ($P<0.05$). This can be explained by the higher amount of maize in the H1 and H2 diet. Maize contains a higher level of xanthophylls, which results in a more orange yolk.

Table 20: The mean values, STD errors and P-values of the different parameters of the physical traits of the eggs

	Diet	Mean	STD error	P-value
Egg weight collection period (g)	C	14.36	0.080	0.217
	H1	14.55	0.080	
	H2	14.49	0.80	
Egg shape (%)	C	75.20[a]	0.21	0.001
	H1	76.27[b]	0.21	
	H2	76.08[b]	0.21	
Surface area (cm^2)	C	26.00	0.11	0.120
	H1	26.30	0.10	
	H2	26.22	0.10	
Egg shell percentage (%)	C	10.12[a]	0.067	<0.001
	H1	10.43[b]	0.073	
	H2	10.52[b]	0.072	
Egg edible portion percentage (%)	C	89.88[a]	0.067	<0.001
	H1	89.57[b]	0.073	
	H2	89.48[b]	0.072	
Albumen pH	C	9.16	0.016	0.374
	H1	9.15	0.16	
	H2	9.18	0.016	
Egg shell thickness (mm)	C	0.2140[a]	0.0012	<0.001
	H1	0.2104[b]	0.0011	
	H2	0.2136[b]	0.0011	
Yolk color	C	4.99[a]	0.070	<0.001
	H1	5.57[b]	0.070	
	H2	5.81[b]	0.061	

a and b – means within parameters with differt letters are significantly different

4.3. Chemical traits of the eggs

Out of tabel 21 can be seen that the different treatments have an effect on the ash level in the eggs ($P<0.05$). There is a significant difference in these levels between C (mean: 1.18 g/100g egg) and H2 (mean:1.36 g/100 g egg). This difference can be explained by the higher levels of phosporus and beta-carotene in the H1 and H2 feed. The found ash concentration is a little bit higher than the one found in the literature study (1.04%).

Also the found concentration of lipids (11.91%) and protein (11.98%) is higher than the concentration found by Dudusola (2010). The dietary treatment has no significant effect on the lipid level of the eggs ($P>0.05$).

Also on the protein level have the treatments an effect ($P<0.05$). H2 has a significantly lower protein level (mean: 1.54 g /100 g egg) than C (mean:12.9 g/100 g egg) and H1 (mean: 12.86 g/100 g egg). If we compare the results of the amino acid profile of Tschriner and Simon (2015) and Degussa (1996) about the BSF and soybean meal, we can see that the BSF larvae has a lower amount of non-essential amino acids in comparison to the soybean meal. The non-essential amino acids have as well a lower percentage, but these can be synthesised in the body, this should not have a big influence on the protein content of the eggs. The essential amino acids can not be synthesised by the body. The lower amount of these in the BSF can explain the lower amount of protein in the eggs. Another factor that explains the lower protein level in the eggs of the H2 diet is the digestibility. Because of the chitin that is present in the BSF, the digestibility is worse than in the control diet. The quails can not digest all the protein which results in a lower protein level in the eggs.

There is no significant difference in the cholesterol content between the three threatments ($P>0.05$). The concentration of oleic and linoleic influence the concentration of the cholesterol in the eggs. The oleic acid level is in the C group higher, but in H1 and H2 is the linoleic acid higher. These levels compensate eachother and results in the same cholesterol content of the eggs.

Table 21: The mean values, STD errors and P-values of the different parameters of the chemical traits of the eggs

	Diet	Mean	STD-error	P-value
DM (g)	C	27.15	0.13	0.147
	H1	27.12	0.10	
	H2	27.48	0.19	
Ash (g/100 g egg)	C	1.18[a]	0.036	0.033
	H1	1.26[ab]	0.046	
	H2	1.36[b]	0.055	
Lipid (g/100 g egg)	C	13.40	0.12	0.159
	H1	13.53	0.092	
	H2	13.76	0.17	
Protein (g/100 g egg)	C	12.90[a]	0.072	0.006
	H1	12.86[a]	0.093	
	H2	12.54[b]	0.072	
Cholesterol (mg/100g egg)	C	499.61	5.99	0.575
	H1	496.81	6.043	
	H2	506.62	7,98	

a and b – means within parameters with differt letters are significantly different

The found FA profile of the eggs is shown in table 22. The main FA in the BSF are lauric acid (C12:0), palmitic acid (C16:0) and oleic acid (C18:1). In the found FA profile of the eggs are the levels of these FA significantly higher as the diet contains more BSF ($P<0.05$). Soybean oil contains a higher amount of palmitic acid and oleic acid than BSF, so a higher amount of these FA was expected in the eggs of the C group.

The amount of MUFA significantly increases as the feed contains higher levels of BSF ($P<0.05$). The different diets do not have an influence of the amount of SFA, PUFA n-6 and PUFA n-3 ($P>0.05$).

Table 22: The mean values (% of total lipid), STD errors and P-values of the different FA found in the quail eggs

Fatty acid	Diet	Mean (%)	STD-error	P-value
SFA				
C6:0	C	0.016	0.0029	0.067
	H1	0.0085	0.0085	
	H2	0.0064	0.0032	
C12:0	C	0.021^{a}	0.0079	<0.001
	H1	0.11^{b}	0.0050	
	H2	0.21^{c}	0.012	
C14:0	C	0.34^{a}	0.0081	<0.001
	H1	1.09^{b}	0.019	
	H2	1.51^{c}	0.040	
C15:0	C	0.053^{a}	0.0014	0.001
	H1	0.051^{a}	0.0005	
	H2	0.062^{b}	0.0022	
C16:0	C	24.19^{a}	0.15	<0.001
	H1	25.62^{b}	0.14	
	H2	26.15^{c}	0.12	
C17:0	C	0.14^{a}	0.0044	0.022
	H1	0.12^{ab}	0.0040	
	H2	0.13^{b}	0.042	
C18:0	C	11.07^{a}	0.11	<0.001
	H1	10.93^{b}	0.12	
	H2	10.21^{b}	0.095	
C20:0	C	0.014^{a}	0.0032	0.028
	H1	0.024^{ab}	0.0010	
	H2	0.026^{b}	0.0018	
C22:0	C	0.083^{a}	0.0047	<0.001
	H1	0.10^{ab}	0.0025	
	H2	0.20^{b}	0.26	
C24:0	C	0.22^{a}	0.0068	<0.001
	H1	0.19^{a}	0.012	
	H2	0.15b	0.011	
ΣSFA	C	36.15	0.51	0.524
	H1	38.25	0.55	
	H2	38.65	0.54	

MUFA				
C14:1	C	0.053[a]	0.0025	<0.001
	H1	0.22[b]	0.010	
	H2	0.36[c]	0.0091	
C16:1	C	2.73[a]	0.95	<0.001
	H1	3.9b	0.097	
	H2	4.54[c]	0.068	
C17:1	C	0.11[a]	0.0021	<0.001
	H1	0.12[b]	0.0026	
	H2	0.13[b]	0.0029	
C18:1	C	31.55[a]	0.53	0.006
	H1	33.17[b]	0.41	
	H2	33.02[b]	0.32	
C20:1 n-9	C	0.13	0.060	0.247
	H1	0.14	0.0058	
	H2	0.14	0.011	
C22:1 n-9	C	0.41	0.0045	0.493
	H1	0.043	0.0028	
	H2	0.037	0.0033	
C24:1 n9	C	0.12	0.0063	0.141
	H1	0.21	0.041	
	H2	0.19	0.024	
ΣMUFA	C	35.10[a]	1.06	0.017
	H1	37.80[ab]	1.16	
	H2	38.42[b]	1.11	
PUFA n-6				
C18:2 n-6	C	19.60[a]	0.39	<0.001
	H1	15.26[b]	0.22	
	H2	13.98[c]	0.35	
C18:3 n-6	C	0.22	0.0077	0.281
	H1	0.20	0.0098	
	H2	0.21	0.011	
C20:2 n-6	C	0.029[a]	0.0043	0.014
	H1	0.034[ab]	0.0051	
	H2	0.046[b]	0.0035	
C20:3 n-6	C	0.098	0.015	0.798
	H1	0.099	0.021	
	H2	0.10	0.025	

C20:4 n-6	C	3.47[a]	0.029	<0.001
	H1	3.32[b]	0.020	
	H2	3.14[c]	0.018	
ΣPUFA n-6	C	23.42	1.24	0.806
	H1	18.91	0.88	
	H2	17.48	0.77	
PUFA n-3				
C18:3 n-3	C	0.48	0.091	0.055
	H1	0.42	0.16	
	H2	0.23	0.032	
C20:3 n-3	C	0.15[a]	0.0050	0.002
	H1	0.18[ab]	0.13	
	H2	0.20[b]	0.014	
C20:5 n-3	C	0.035	0.0033	0.383
	H1	0.040	0.0019	
	H2	0.038	0.0024	
C22:6 n-3	C	1.28[a]	0.016	<0.001
	H1	1.00[b]	0.040	
	H2	0.69[c]	0.013	
ΣPUFA n-3	C	1.95	0.77	0.695
	H1	1.64	0.67	
	H2	1.16	0.37	

a, b and c – means within fatty acids with differt letters are significantly different

4.4. Shelf-life performances

The amount of MDA is the same on day 0 in the different treatments (P>0.05) as can be seen in table 23. The C group has the lowest amount of MDA (mean: 1.72 mg/kg egg). On day 28 the C is significantly higher in MDA than the H1 group (P<0.05).

It would be expected that the amount of MDA increases as time passes, but here this is not the case. The following aspects can explain these data. First of all, the variation in the MDA levels stays the same or has a small change over time. This small change does not have a lot of meaning. The decrease of MDA in H1 and H2 can be explained by the fact that the same reagents were used at day 0 and day 28. During this period, the reactivity to the TBARS could have been slightly reduced. There was no other choice than to use the same reagents because that way a different calibration curve would have been obtained.

We can assume that the eggs have a very long shelf-life, this is because the physical form of the egg. The content is closed of from the environment by the eggshell.

Tabel 23: The mean values, STD errors and P-values of the amounts of MDA on day 0 and 28

		Mean	STD-error	P-value
TBARS day 0 (mg MDA/kg egg)	C	1.72	0.011	0.077
	H1	1.82	0.020	
	H2	1.78	0.046	
TBARS day 28 (mg MDA/kg egg)	C	1.72[a]	0.013	0.001
	H1	1.60[b]	0.012	
	H2	1.66[ab]	0.03	

a and b – means within parameters with differt letters are significantly different

4.5. Life performances

The results found of the life performances of the quails are shown in table 24. The amount of eggs per diet do not differ significantly (P>0.05). In cage 6A (H2) the amount of eggs is remarkably lower (269 eggs in total) than in the rest of the cages. A possible explanation is the mortality in that cage. Two quails died, it is possible that they died in the beginning of the trial and pulled the amount of eggs per day down. Although there is probably another explanation because in cage 2B and 3A respectively 3 and 2 quails died and there is no remarkable low amount of eggs these cages. Possibly in 6A something went wrong with the feed intake, the food could have been difficult to reach which results in a lower feed intake, which results in fewer eggs.

The laying performance (73.03%) is comparable to the one found in the literature study (75%). The egg weight of C is significantly lower than the egg weight of H1 (P<0.05), but in the week of the collection the egg weights do not differ anymore. The difference in egg weight in the first week can be explained by an adaptation to the feed.

The quails weight does not differ significantly between the different diets (P>0.05), there is also no difference found on cage base (P>0.05). The quails weigh less at the end of the experiment, this can be explained by their age. In the beginning they are 3 months old, they reached their maximum weight at this time. Each cage had possibly approximately the same energy intake. The dead quails are not calculated in the final weight, these are very light. Possibly if these stayed alive, the mean weight per cage would not have been the same.

The H1 has the heighest number of deaths, namely seven, al these quails have a very low weight, their weight is lower than the weights of the dead quails from C and H2. These findings can not be explained. In table 25 an overview of the mortality of the quails is given.

Table 24: The mean values, STD errors and P-values of the different parameters for the live performances of the quails

Parameter	Diet	Mean	STD-error	P-value
Total amount of eggs	C	391.60	14.33	0.881
	H1	380.60	2.00	
	H2	378.60	28.11	
Amount of eggs per day	C	11.19	0.41	0.881
	H1	10.87	0.34	
	H2	10.82	0.80	
Egg weight total period (g)	C	14.33[a]	0.029	<0.001
	H1	14.66[b]	0.031	
	H2	14.48[c]	0.033	
Quail initial weight (g)	C	364.44	4.72	0.858
	H1	367.06	4.12	
	H2	365.89	5.94	
Quail final weight (g)	C	351.96	5.94	0.519
	H1	360.65	5.32	
	H2	356.11	4.78	

a and b – means within parameters with differt letters are significantly different

Table 25: Weight of found dead quails in different cages

Diet	Cage	Weight (g)
C	1A	337
C	1B	303
C	4B	323
H1	2A	246
H1	2B	174
H1	2B	252
H1	2B	149
H1	5A	195
H1	5B	142
H1	5B	179
H2	3A	254
H2	6A	301
H2	6A	303
H2	7A	207

5. Discussion

The sensory analysis showed that there is no difference in taste, the insect diets do not give an odd taste to the eggs which is an important aspect regarding the acceptation by the custumor. Al-Qazzaz et al. (2016) did see a significant improvement of the appearance, texture and the taste of eggs with increasing levels of BSF in the feed. He found also no difference in egg odor. The consumers preferences depend on the different diets regarding the yolk colour. A different yolk colour is found in the different diets. Preferences of the yolk colour are linked to geographical location, culture and traditions. A survey showed that Europe prefers more dark colours (Beardsworth et al., 2004), in this regard the eggs of quails fed with H2 are preferred by the consumer. On the contrary, it is hard to notice the colour difference without the Roche Yolk Colour Fan or without seeing the yolks of the different diets next to each other. Because of this the difference in the yolk colour will not have a great influence on the consumers behavior. The found yolk colour score of the C diet is comparable to the one reported by Zita et al. (2013), the scores of H1 and H2 are higher. Zita et al. (2013) did not describe the feed used in the experiment which affects the yolk color .

Another important aspect for the consumer and the sellers of the product is the shelf-life. The eggs in this trial did not deteriorate during 28 days, they have a long shelf-life. This is seen by the amount of mg MDA/kg egg. Also the pH of the albumen can say something about the storage. How longer eggs are stored, the more carbon dioxide (CO_2) is lost through the shell pores, and the higher the pH gets. This can be accelerated by a higher storage temperature. This process depends on dissolved CO_2, bicarbonate ions, carbonate ions and protein equilibrium (Goodrum et al., 1989). Since the eggs have the same shelf life, the amount of mg MDA/kg egg is the same throughout treatments, it was expected that the differents diets resulted in the same pH of the albumen.

All-Qazzaz et al. (2016) found a lower egg weight with hens fed with a diet that contained 50 g kg^{-1} in comparison to a diet with no BSF and 10 g kg^{-1}. This was explained by the energy level in the diets, when the energy level decreased from 2800 to 2700 kcal kg^{-1}, the egg weight decreased from 47.66 to 46.41 g. Widjastuti et al. (2014) showed that the substitution of fish meal by maggot meal of BSF until 50% resulted in a higher egg weight. These results were explained by a higher feed intake of the diets when 25 and 50% of the fishmeal was replaced by BSF maggot meal. In this experiment there is no difference in egg weight, although the diets are not isocaloric. Possibly there was a higher feed intake with H1 and H2 which resulted in a comparable energy intake in the 3 diets and the same egg weight.

The shell weight increases as the level of BSF increases. This is not expected since the egg shell thickness is the highest in the C group and shell weight and thickness are positively correlated. The surface area does not change significantly between treatments, a bigger surface area in H1 and H2 could have explained the higher shell weight, but here this is not the case. Maybe a mistake in the measuring of the shell weight did occur. Possibly the shell of H1 and H2 were not dried as good as these of C, but this is unlikely since the eggs between different treatments were alternated while measuring. If the egg shell weight in C was higher than in H1 and H2 the egg shell percentage and egg edible portion percentage would have shown different results, these would respectively be higher and lower in the C group in comparison to H1 and H2. The higher shell weight in the C group was mainly expected because of the higher level of Ca in the C diet. The egg shells consist mainly of Ca, so a lower amount of calcium in the diet will have an effect of the formation of the egg shell. Al-Qazzaz et al. (2016) found a lower egg shell weight and thickness with increasing levels of BSF. Sari et al. (2016) reported an egg shell weight of 1.30 g, while Zita et al. (2013) found an shell weight of 1.06 g. Both of them did not describe the amount of Ca in the feed. The values found in this experiment are higher.

The higher ash content found in H1 and H2 is due to the higher amount of phosporus in these diets. The found ash content is higher than the one reported by Dudusola (2010). This variation can possibly be explained by different feed or different genetics. The used feed in the experiment is not reported.

The lower protein level in the eggs when BSF is added to the diet can be fixed by changing the diet. Adding chitin degrading enzymes or bacteria to the feed can improve the digistibility, resulting in a higher protein level in the eggs. Also the addition of essential amino acids can increase protein value of the diet.

No significant change in the lipid level is found between the different treatments, but the fatty acid profile is different. The quails fed with H2 have a significantly higher level of MUFA than C in the eggs, this difference is mainly because of the higher levels of palmitoleic and oleic acid. MUFA can lower LDL-cholesterol, but does not lower HDL cholesterol, which is beneficiary for human health. A reduction in total and LDL cholesterol results in a lower incidence of cardiovarsculair diseases (Kris-Etherton, Pearson et al., 1999).

The daily laying performance of the quails with the three different diets stays the same. The farmer will be satisfied equally with all three diets, they lay an equal amount of eggs and the eggs have the same weight. But if we look at our presumption that H1 and H2 maybe had a higher feed intake, the farmer will prefer the C diet. An adaption of the feed formula can be made which results in a higher energy content of H1 and H2, this way it could be that there will not be a higher feed intake of these diets.

A problem found is the mortality, the mortality in H1 is a lot higher than in C and H2. The dead quails of H1 were lighter than the other dead quails. An explanation can be sought in the feed intake, possibly the feed was unattractive. Although this is probably not the case here because this contradicts other presumptions and the living quails of the H1 group have a normal body weight. Also, in the H2 group the weights of the dead quails did not differ a lot from the ones of the C group. If we saw that in the H2 group, a higher number of quails died and also these were very light, than we could assume that there was a problem with the intake or the feed, of the feed itself for example a shortage of essential nutrients. But here this is not the case. The lower weight of these quails can be a coincidence, as this is a trial with living creatures, not every variance can be explained.

6. Conclusion

The inclusion of BSF up to 15% in laying quails feed resulted in a good egg quality and live perfomances of the quails.
The consumer will only be able to distinguish the eggs resulting from the different diets on yolk colour since there is no detected difference in taste. Possibly, he/she will not notice the difference in yolk colour.
Also for the farmer the BSF is a good substitution in the quail feed. There is no difference in egg weight, laying performance and shelf-life.
If we compare the chemical analysis of the different diets, the results are promising. An inclusion of BSF caused an increase of the ash level, but a lower protein level. Chitin obstructs the digestion of protein, by adding chitin-degrading enzymes to the feed, the protein in the feed will be easier digested which results in a higher protein level in the eggs. The same amount of fat is found in all the eggs, but the FA profile differs. Quails fed with BSF lay eggs with a higher level of MUFA, which is beneficiary for human health.
The higher amount of dead quails with a lower body weight in the H1 group and the low amount of layed eggs in cage 6A is assumed to be a coincidence, as some occurings can not be explained in an animal trial.

We can conclude that BSF is a suitable feed ingredient for laying quails as the inclusion up to 15% results in a good egg quality and live performance of the quails.

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